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Phagocytosis of spermatozoa by epithelial cells in the vagina of the lizard *Hemidactylus mabouia* (Reptilia, Squamata)

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ABSTRACT

Hemidactylus mabouia is an Africa oviparous lizard that is now distributed on other continents and has been introduced to Brazil. In the majority of reptiles, the females have the ability to store spermatozoa in specialized regions of the genital tract. Considering that in *H. mabouia* the storage of spermatozoa is restricted to the region of the uterine tube, in this study we utilized optical and transmission electron microscopy to investigate the processes related to the large number of spermatozoa in the vagina. Although it was possible to visualize spermatozoa in the vagina, an ultrastructural analysis of the region revealed that significant phagocytosis occurs, which is mediated by the epithelial cells. Such a process indicates that the anterior portion of the vagina is related to the elimination of supernumerary or deficient spermatozoa and not storage.

1. Introduction

In the majority of vertebrates, during mating, part of the spermatozoa deposited in the female reproductive tract does not reach the fertilization site. The residual spermatozoa are eliminated from the female reproductive tract by phagocytosis (Chakraborty and Nelson, 1975; Koyanagi and Nishiyama, 1981; Matthijs et al., 2000; Eisenbach, 2003; Suarez and Pacey, 2006). Phagocytosis of spermatozoa in the female reproductive tract is characterized as a slow process beginning with the formation of pseudopodia, followed by the consumption of entire spermatozoa or their fragments (Mori and Uchida, 1974; Murakami et al., 1985; Phillips and Mahler, 1977a,b).

The sites of phagocytosis vary between species; in lizards, its occurrence has been reported in storage locations, such as those in the uterine tube (Sever and Hopkins, 2004) and vagina (Siegel and Server, 2007). Spermatozoa were reported to be present in the vagina of the lizards *Eumeces egregius* and *Hemidactylus frenatus* (Schafer and Roeding, 1973; Eckstut et al., 2009); however, this region was not specified as the location of phagocytosis or storage due to the absence of specialized structures for this function, such as crypts.

In the oviparous lizard *Hemidactylus mabouia*, the storage of spermatozoa occurs in the uterine tube. This region and the infundibulum, which opens into the coelomic cavity, are the probable locations of fertilization in reptiles (Girling, 2002). Despite some studies describing the phagocytosis of spermatozoa in sites of storage and fertilization (Sever and Hopkins, 2004; Siegel and Server, 2007), no reports were found on the occurrence of this process in other regions of the female reproductive tract. Considering the presence of spermatozoa in the vaginal region, we characterized in this study the structure and ultrastructure of this region, searching for explanations for the large number of these cells outside the storage site.

2. Materials and methods

2.1. Animals

Six female *H. mabouia* lizards were collected under license number 10504-1 of IBAMA, and the carcasses were fixed in a buffered formalin solution (Carson et al., 1973) for 24 h at room temperature and deposited at the João Moojen Animal Science Museum of the Universidade Federal de Viçosa (UFV), Brazil. The experiment was conducted in accordance with the ethical principles for the utilization of laboratory animals published in the Colégio Brasileiro de Experimentação Animal – COBEA. The snout-vent length

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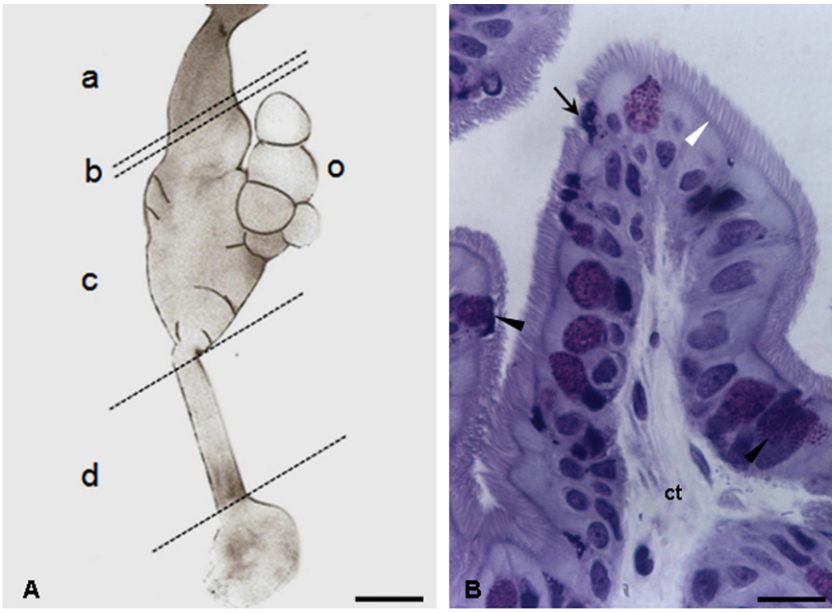

Fig. 1. (A) Scheme of the right portion of the female reproductive system of *H. mabouia*. a, infundibulum; b, uterine tube; c, uterus; d, vagina; o, ovary. Bar: 1.5 mm. (B) Light microscopy of the anterior portion of the vagina. Arrow, phagocytic cell; white arrow head, ciliated cell; black arrow head, secretory cell; ct, connective tissue. Bar: 20 μm.

of the specimens utilized varied between 51 and 57 mm and is in accordance with the criteria established by Rocha et al. (2002) for their characterization as adults. Identification and collection of the reproductive organs were done *in situ* via a median longitudinal incision in the ventral region of the animals in a saline solution (NaCl 0.9%).

2.2. Optical microscopy

Fragments of the vagina, approximately 4 mm, were fixed in a buffered formalin solution (Carson et al., 1973) for 24 h at room temperature and were then dehydrated in increasing concentrations of ethanol. The fragments were embedded in glycol methacrylate (Historesin®, Leica) oriented cross-sectional and longitudinal and sectioned at 2 μm in an automatic microtome (Reichert-Jung, Germany). The obtained sections were stained with 1% toluidine blue/sodium borate and mounted on slides with Entellan® (Merck) for analysis with an Olympus BX-60 microscope

2.3. Transmission electron microscopy

Fragments of the vagina, approximately 1 mm^2, were fixed in Karnovsky's solution (Sheehan and Hrapchak, 1980) for 4 h. The material was next washed in 0.1 M sodium cacodylate buffer (pH 7.2) and post-fixed in 1% osmium tetroxide for 2 h. Subsequently, the material was dehydrated in increasing series of acetone and embedded in Epon resin. Ultrathin sections were obtained in an ultramicrotome and contrasted stained with 2% uranyl acetate and 0.2% lead citrate. The material was examined and photographed in an EM 109-Zeiss transmission electron microscope.

3. Results

The female reproductive system of *H. mabouia* is composed of the ovaries and oviducts. Each oviduct is formed by four histologically distinct regions, known as the infundibulum, the uterine tube, the uterus and the vagina (Fig. 1A) (Girling, 2002; Nogueira, 2008).

The anterior portion of the vagina is composed of a muscular tube internally lined with a simple prismatic epithelium containing ciliated cells and two types of non-ciliated cells (Figs. 1B and 2A). An ultrastructural analysis showed that the non-ciliated cells may be characterized as secretory and phagocytic (Fig. 2A).

The ciliated cells predominate and possess long cilia with well-developed basal bodies. The nucleus has an oval shape and is located in the basal third of the cell. The numerous mitochondria are concentrated in the supranuclear region (Fig. 2A).

Secretory cells have a rounded nucleus near their base and extensive rough endoplasmic reticulum. The apical third of the cell is filled by large secretory granules with different densities (Fig. 2A).

Phagocytic cells differ from the other cell types principally by not possessing cilia or secretion granules (Fig. 2A). Mitochondria and rough endoplasmic reticulum are less evident when compared to the other epithelial cells (Fig. 2A). The apical portion of the phagocytic cells extends large projections that engulf groups of spermatozoa (Fig. 3A).

In the cytoplasm, near the phagosomes containing spermatozoa, small oval-shaped electron-lucent vesicles measuring roughly 0.4 μm along their greatest axis were observed (Fig. 3B). Each vesicle had one eccentrically located electron-dense granule in its interior (Fig. 3B).

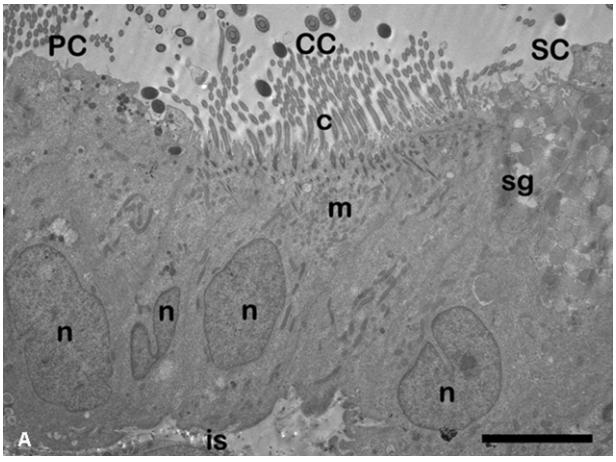

Fig. 2. (A) Transmission electron microscopy of the anterior portion of the vagina. PC, phagocytic cell; CC, ciliated cell; SC, secretory cell; c, cilia; is, intercellular space; m, mitochondria; n, nucleus; sg, secretory granule. Bar: 5 μm.

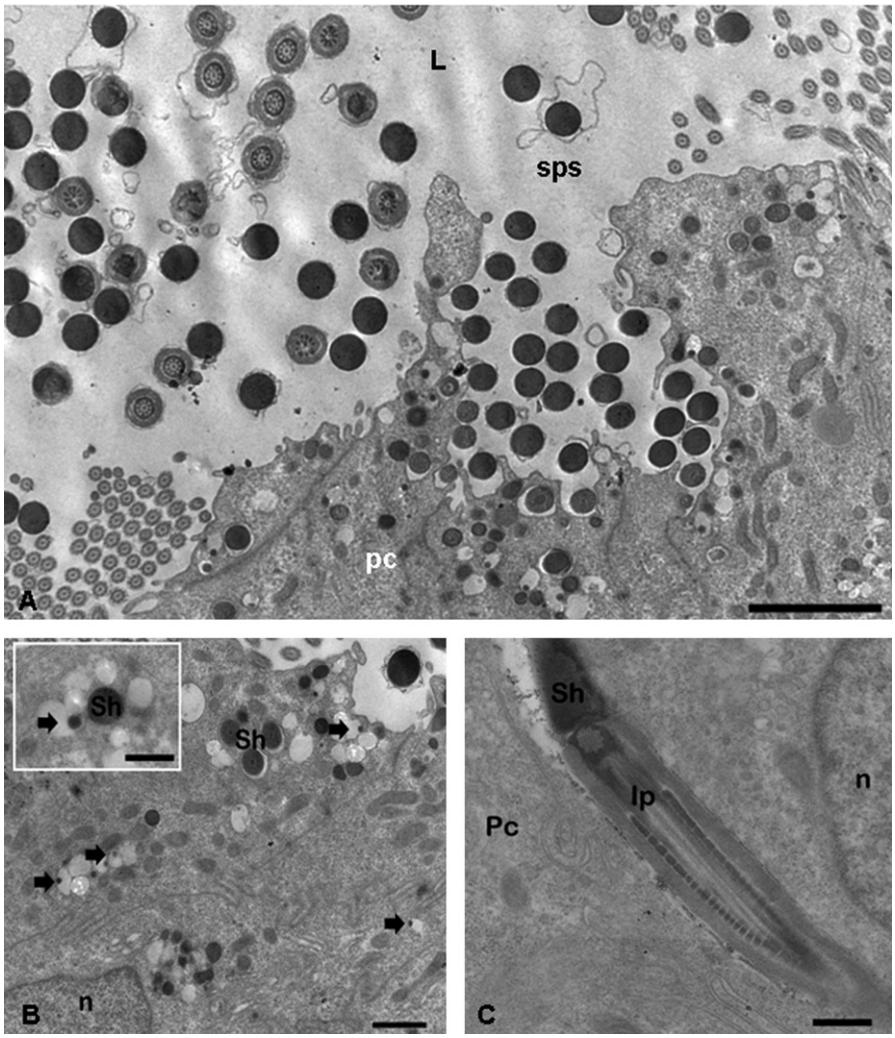

Fig. 3. (A) Note the apical projections of phagocytic cells (pc) evolving groups of spermatozoa (sps), seen in transverse sections. L, lumen. Bar: 2 μm. (B) Imminent phagocytosis. Presence of many vesicles with characteristics of lysosomes (arrows) in the cytoplasm of phagocytic cells. Note the vesicles abridging engulfed spermatozoon (insert). n, nucleus; Sh, sperm head. Bar: 0.6 μm. (C) Spermatozoon positioned longitudinally in the cytoplasm of phagocytic cells. Ip, middle piece; n, nucleus; Pc, phagocytic cell; Sh, sperm head. Bar: 0.25 μm.

Sometimes the material in the digestion process can be identified as fragments of the heads and tails of the spermatozoa, indicating that these cells are completely engulfed (Fig. 3A and C). The majority of spermatozoa are engulfed along their longitudinal axis; however, spermatozoa in other orientations were observed inside the cells (Fig. 3C).

4. Discussion

Phagocytosis of spermatozoa by epithelial cells of the female reproductive tract has been described in various vertebrate taxa, including mammals (Eisenbach, 2003; Suarez and Pacey, 2006), birds (Koyanagi and Nishiyama, 1981), amphibians (Sever and Brunette, 1993) and reptiles (Sever and Hopkins, 2004). In reptiles, this process has only been observed in locations of storage (Sever and Hopkins, 2004), as in amphibians, where phagocytosis and the cells responsible for this process were reported exclusively in the spermatheca. In *H. mabouia*, the spermatozoa present in the vaginal region, which is not characterized as a storage site, are phagocytosed by epithelial cells. In other groups, such as in birds, the spermiophagy process appears to be executed by intra-epithelial macrophages (Koyanagi and Nishiyama, 1981). There is evidence that spermiophagy of the female reproductive tract is a universal process among mammals (Murakami et al., 1985); however, observations in *H. mabouia* and other taxa indicate that this hypothesis may be extended to all animals with internal fecundation.

Studies of the morphology of the oviduct in reptiles have described the ciliated and non-ciliated cells of the vaginal epithelium (Girling et al., 1997, 1998; Girling, 2002; Sever et al., 2000). However, these studies considered the non-ciliated cells to function solely as secretory cells. In this study, we showed that there are two non-ciliated cell types in *H. mabouia*, one secretory type commonly reported for reptiles and the other type characterized as an epithelial phagocytic cell. The epithelial phagocytic cells were first reported in the oviducts of mammals (Austin, 1960) and possess typical morphological characteristics different from those characterized for the phagocytes (Sever, 1992).

Many of the spermatozoa phagocytosed by the epithelial phagocytic cells of *H. mabouia* were enveloped by vesicles in the cytoplasm. It is possible that the vesicles containing electron-dense granules are lysosomes because they are observed grouped around material in the process of being digested. The phagocytosed spermatozoa may present defects that were not observed in morphological analyses; the anterior vagina is believed to be the location of selection, in which spermatozoa without fertilization capacity are eliminated before they can generate an inflammatory response, as observed in mammals (Eisenbach, 2003).

Some Squamata are capable of storing spermatozoa in the vagina; however, the ultrastructural observations in *H. mabouia* eliminated this possibility because the presence of specialized structures is necessary for storage, which guarantees an increase in the motility and survival of the spermatozoa (Olsson and Madsen, 1998). Phagocytosis outside the storage site in *H. mabouia* possibly has the function of eliminating supernumerary spermatozoa that do not reach the fertilization site, which explains the large number of these cells observed in the anterior portion of the vagina.

Acknowledgments

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